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Related Patents

LETTERS PATENT

Number **540594**

ELIZABETH THE SECOND, by the Grace of God Queen of New Zealand and Her Other Realms and Territories, Head of the Commonwealth, Defender of the Faith; To all to whom these presents shall come, Greeting:

WHEREAS pursuant to the Patents Act 1953 an application has been made for a patent of an invention for

Method for inhibiting mycotoxin contamination in cereals

(more particularly described in the complete specification relating to the application)

AND WHEREAS

Sankyo Agro Company, Limited, 23-14, Hongo 4-chome, Bunkyo-ku, Tokyo 113-0033, Japan
Hokkai Sankyo Co., Ltd., 1, Ohdori Nishi 8-chome, Chuo-ku, Sapporo-shi, Hokkaido 060-0042, Japan

(hereinafter together with his or their successors and assigns or any of them called "the patentee") is entitled to be registered as the proprietor of the patent hereinafter granted:

Address for service: BALDWINS, Level 14, Baldwins Centre, 342 Lambton Quay, Wellington, New Zealand

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IN WITNESS whereof We have caused these letters patent to be signed and sealed on 10 July 2008 with effect from 4 December 2003.

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NEW ZEALAND

Neville Harris
Commissioner of Patents, Trade Marks and Designs

540594

Specification

Method for inhibiting mycotoxin contamination in cereals

Technical Field

The present invention relates to a treatment method which intends to reduce the amount of mycotoxin contamination where mycotoxin is produced by plant pathogenic fungi in cereals (hereinafter represented as "DON") by using a composition for agri-horticulture containing one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester as an active ingredient(s).

Background Art

Fusarium head blight in cereals frequently occurs in cases of continuous cloudy weather and light rain, and furthermore in conditions of high temperature from the heading date to the milk-ripe stage (Yukio Ozeki, Hiroshi Sasaki, Yoichi Amano, Hokkaido no Hatasaku Gijutu (Farming Technology in Hokkaido) -Cereals version-, published by Society for Agricultural Technique Propagation, page 209, 1978), and it is a plant disease which causes great damage to cereals in terms of yields, quality and so forth, and can not be avoided in this country due to the high amount of rainfall during the ripening period. As major pathogenic fungi thereof, *Fusarium graminearum*, *Fusarium culmorum*, *F. avenaceum* and *Microdochium nivale* have been specified. Although there is a difference in occupancy depending on climate conditions and areas, complex infection is often observed in farm fields where the disease has occurred (Kunihei Kishi, Nippon Shokubutsu Byogai Daijiten (Comprehensive Dictionary of Plant Disease in Japan), published by Zenkoku Noson Kyoiku Kyokai

(Association for National Farming Village Education), page 74, 1998).

The plant pathogenic fungi which cause this disease produce more than one toxic metabolite referred to as mycotoxin, which contaminates crops during cultivation and poses a risk of ingestion by human and domestic animals through migration to harvested and processed foods. There is a long history of research on mycotoxin in areas such as Europe, North America and East Asia where cereal cultivation is active and DON has been specified as a toxic agent whose effects on humans and animals are most concerning in terms of both toxicity and the amount of contamination, and is noticed worldwide. The ingestion of foods contaminated with DON causes acute poisoning of which major symptoms are digestive organ symptoms such as emesis and diarrhea. In Europe and North America, self-regulating values of DON contamination in grains have been established, and a system for intensified surveillance has been organized. While there is increasing international momentum in DON surveillance, in this country in 2002, the Ministry of Health, Labor and Welfare also established an interim standard value of 1.1 ppm for DON contamination in wheat, and announced that the safety of the wheat distributed in the market was assured (Shokuhatu No. 0521001). In relation to this announcement, as an instruction notice for feed safety, the Ministry of Agriculture, Forestry and Fisheries established the interim acceptable value of 4.0 ppm for deoxynivalenol in feeds supplied to cattle aged 3 months or more and 1.0 ppm for domestic animals other than the above (press release). Before DON has been noticed as a toxin produced by fungi, people have been protected from health risks caused by fungous toxins by a law regulation that the contamination ratio of mixed grains affected with Fusarium head blight shall be less than 1% by visual check at the shipping stage of crude wheat. Thus, in the cereal production fields,

Fusarium head blight damage has been reduced and inhibited by applying fungicidal agents which are effective against pathogenic fungi which cause Fusarium head blight.

Commonly used chemicals at the present time which are effective against the pathogenic fungi which cause Fusarium head blight in cereals are presently classified into several groups by their mechanisms of action and the chemical structures of the active ingredients (Agricultural Chemical Handbook, 2001 version, published by Nippon Shokubutsu Boeki Kyokai). The SBI agents are characterized by inhibiting the biosynthesis of sterols which are universally present as a component of the biological membrane of fungi and include (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazole-1-ylmethyl) pentane-3-ol (common name: tebuconazole), (1RS, 5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1-H-1,2,4-triazole-1-ylmethyl) cyclopentanol (common name: metconazole), and 1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolane-2-ylmethyl)-1H-1,2,4-triazole (common name: propiconazole), which are characterized by having a triazole skeleton in their chemical structure. They have been widely used because they are effective at low doses, are resistant to rain due to rapid permeation of plant bodies, and have low toxicity against flower-visiting insects, and they also have a high controlling effect against the pathogenic fungi which cause Fusarium head blight in cereals. Methyl=(E)-2-{2-[6-(2-cyanophenoxy) pyrimidine-4-yloxy]phenyl}-3-methoxyacrylate (common name: azoxystrobin) and methyl=(E)-2-methoxyimino [α-(o-tolyloxy)-o-tolyl] acetate (common name: kresoxim-methyl), which were developed as strobilurin derivatives (which are anti-fungal antibiotics and structurally characterised by methoxyacrylate ester) are classified as methoxyacrylate type fungicidal agents. The former exerts a controlling effect by inhibiting the respiratory activity of fungi, and the latter exerts a controlling effect by inhibiting the cytochrome electron transfer system in

mitochondria. The other synthetic fungicidal agents include 1,1'-iminodi(octamethylene)diguanidium=triacetate (common name: iminoctadine acetate). It is considered that the mechanism of action of this compound is to destroy the membrane lipid bilayer structure of fungi based on a surfactant-like feature derived from its salt structure. Furthermore, the relationship between pathogenic fungi and drug efficacy has been researched, and it has been demonstrated that triazole agents are effective against the 3 species of *F. graminearum*, *F. culmorum* and *F. avenaceum*, and that the methoxyacrylate agents are effective against *M. nivale*. As mentioned above, occurrence of this disease is often associated with a mixture of several pathogenic fungi, and thus, the occurrence of this disease has been prevented and inhibited by spraying the respective agents in rotation and using their characteristics.

Along with attention to DON, its contamination concentration has been analysed, and then it has been demonstrated that there is no direct correlation between an illness-inducing degree of Fusarium head blight and the level of DON contamination (Bai, G. H., Plattner R and Desjardins A., Relationship between visual scab rating and deoxynivalenol in wheat cultivars, The 1988 National Fusarium Head Blight Forum, Chapter 2, pages 21-25). It has also been demonstrated in recent years that *F. graminearum* and *F. culmorum* have DON producing capacity but *F. avenaceum* and *M. nivale* do not produce DON. It has been reported that when *M. nivale* is controlled using the methoxyacrylate agent, competitive *F. graminearum* and *F. culmorum* increase, resulting in facilitating an increase in DON contamination. Thus, controlling disease by applying fungicidal agents alone does not sufficiently prevent DON contamination. Furthermore, in cereal production fields, even when cereal Fusarium head blight is controlled by the combined application of various fungicidal agents, DON is frequently detected at more than 1.1 ppm. This troubles

the producers. That is, DON contamination can not be sufficiently reduced by the conventional method of controlling mycotoxin producing pathogenic fungi alone. Also with respect to fosetyl described in the present application, the fungicidal effect against plant pathogenic fungi is publicly known (US Patent No. 4,139,616, 1979; and JP 62-87504 A), but there is no mention at all of the effects of fosetyl on plants contaminated with mycotoxin. Under such circumstances, the establishment of a method to substantially inhibit DON contamination in cereal producing fields is strongly desired in the present situation.

The present inventors studied compositions used in agriculture which inhibit mycotoxin contamination in wheat, particularly inhibiting DON contamination to 1.1 ppm or less or as low as they possibly can. As a result, they have found that a composition for agri-horticulture developed using one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester as an active ingredient(s), has a weak controlling effect against pathogenic fungi which cause Fusarium head blight in cereals, but has an excellent inhibitory effect on mycotoxin contamination, particularly DON contamination, and have completed the invention.

Also, the inventors have shown a reduction in the DON contamination level equal to or less than the standard value when compound A is used in combination with a fungicidal agent where DON contamination has been observed at a high concentration of more than the standard value of 1.1 ppm, and a further inhibitory effect on DON contamination when compound A is used in combination with a fungicidal agent which originally lowers the contamination level, and have found the combination further inhibits DON contamination when compared to a treatment with a fungicidal agent for agri-horticulture alone and have

completed the invention.

Disclosure of the Invention

The present invention is a composition containing one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester as an active ingredient(s) when used to inhibit mycotoxin contamination (particularly, deoxynivalenol) in cereals.

Also, the invention relates to a method for inhibiting mycotoxin (particularly, deoxynivalenol) contamination in cereals wherein a composition containing one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester as an active ingredient(s) is applied to the cereals.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 represents the test results of the inhibitory effects of phosphite derivatives and alkyl phosphite derivatives on DON contamination (Example 1).

Fig. 2 represents the test results of the inhibitory effects of potassium phosphite used in combination with various fungicides (Example 3).

Best Mode for Carrying Out the Invention

Hereinafter, the invention is described in detail.

The ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester derived from phosphorous acid are not particularly limited as long as they inhibit mycotoxin contamination,

particularly DON contamination. These compounds include, for example, alkali metal salts and polyvalent metal salts of phosphorous acid and phosphite ester, and are suitably potassium phosphite and an aluminium salt of tris(ethylphosphonate)(common name: fosetyl).

Methods of assessing the inhibitory effect of the invention on mycotoxin contamination, particularly DON contamination in wheat, can include a method of determination, or measuring, by comparing DON concentrations, the incidence rate of Fusarium head blight for panicles and the incidence rate for spikelets in wheat cultivated in a field between a group where a treatment used with phosphorous acid and a phosphite ester derivative alone was applied, and a group where a treatment used in combination with other fungicidal compositions was applied, and a control group without drug treatment.

It has been confirmed by such a method that the ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester derived from phosphorous acid have an excellent inhibitory effect on mycotoxin contamination, particularly DON contamination, which is independent of any ability to control pathogenic fungi in cereals.

The compound B in the invention could be a usual fungicidal active compound for agri-horticulture, and is suitably a fungicidal active compound which is effective against wheat Fusarium head blight, such as a sterol biosynthesis inhibitor (SBI) having a triazole skeleton, azoxystrobin, kresoxim-methyl and iminoctadine.

EXAMPLES

By citing test examples below which use potassium phosphite and an aluminium salt of tris(ethylphosphonate) (common name: fosetyl) as the compounds A, which are the active ingredients of the invention, the invention is more

specifically described, but the invention is not limited thereto.

Example 1: Effect used alone (± conventional control)

Wheat (cultivar: Haruyutaka) was seeded on April 19, 2002, and cultivated according to a conventional cultivating standard (Hokkaido, Agriculture Department, 1995) to establish test plots with 6.75 m^2 per plot (3 repeats). As the compounds A, potassium phosphite and an aluminium salt of tris(ethylphosphonate)(common name: fosetyl) were applied, each of their aqueous solutions of 0.038 to 0.120 % as P_2O_5 was prepared, sprayed on leaves at the next growth stage at a rate of 100L/10a. That is, a first spray (June 28, flowering date), a second spray (July 8, 10 days after the flowering date), and a third spray (July 18, 20 days after the flowering date) were performed. A conventional control for the purpose of controlling plant diseases and plant insects was performed in combination with the above sprays. That is, a first spray [June 20, azoxystrobin (2000 times dilution) + fenitrothion (1000 times dilution)], a second spray [tebuconazole (June 28, 2000 times dilution) + sumithione (1000 times dilution)], a third spray [July 8, propiconazole (2000 times dilution) + fenitrothion (1000 times dilution)], and a fourth spray [July 17, tebuconazole (2000 times dilution) + fenitrothion (1000 times dilution)] were performed. As controls, a test plot where only the conventional control was given and a test plot without any control were established. Harvest was performed by harvesting the wheat in 4 m^2 of the test plots on August 9 (52 days after the heading date). After harvesting, the wheat was applied to a rice grader with 2.2 mm screen openings to sieve selected grains, which were then pulverized by a high-speed pulverizer to make whole grain powder. The whole grain powder was used as a sample for analysis. The DON contamination concentration was analyzed by ELISA using a commercially available Rida Screen Fast DON supplied by r-Biopharm.

The preparation of analysis sample solutions and the analytical procedure are briefly described.

- 100 ml of water was added to 5 g of the whole grain powder, and vigorously agitated for 10 min to make a DON extract solution.

- The DON extract solution was centrifuged at high speed, and the supernatant was used for ELISA analysis.

- According to the method described in the ELISA kit, various reagents were added, and subsequently the absorbance of each test solution was measured.

- The DON concentration of each test solution was read out from a standard curve made using DON standard solutions.

- The incidence rate of Fusarium head blight for panicles was calculated by counting the number of panicles in 1 m^2 of each plot and the number of diseased panicles included therein.

The results of the present test are shown in Table 1.

Table 1. Inhibitory effect of a phosphite derivative and an alkyl phosphite derivative on DON contamination (2002, Haruyutaka)

	Treatment			DON concentration (ppm)	Incidence rate of Fusarium head blight for panicles (%)
	Active ingredient A	Concentration	Control system		
1	Potassium phosphite	0.038%	Conventional control	2.41	0.3
2		0.070%	Conventional control	0.59	0.0
3		0.112%	Conventional control	0.39	0.9
4			No control	0.69	1.2
5	Fosetyl	0.120%	Conventional control	0.84	0.0
6	Potassium phosphate	0.112%	Conventional control	4.66	0.0
7			Conventional control	6.16	0.9
8			No control	8.69	4.9

Note: The concentration of the active ingredient A is the concentration converted into P_2O_5.

Note: The incidence rate of Fusarium head blight for panicles was calculated by counting the number of panicles in 1 m^2 of each plot and the number of diseased panicles included therein.

High levels of DON contamination, which were much higher than the interim standard value of 1.1 ppm set by the Ministry of Health, Labor and Welfare, were detected in the plot with no control and in the plot with conventional control of a fungicidal composition for controlling Fusarium head blight in cereals. On the other hand, concentration-dependent inhibition of DON contamination was observed in the plots treated with potassium phosphite and the aluminium salt of tris(ethylphosphonate)(common name: fosetyl), and their contamination levels were lower than 1.1 ppm in these plots. It is worthy of special mention

that even in the plot without any control, where the conventional control was not performed, the DON contamination concentration was sufficiently inhibited by applying potassium phosphite. Furthermore, even when potassium phosphate which is a salt of orthophosphoric acid is applied, the DON inhibitory effect is low, and thus it is obvious that the phosphite and alkyl phosphite derivatives play an important role. Even when the incidence rate of Fusarium head blight for panicles was at almost the same degree, high concentrations of DON were detected in the plot without potassium phosphite treatment. Therefore, it is evident that DON contamination is inhibited by treatment with phosphorous acid and alkyl phosphorous acid and derivatives thereof.

Example 2: Toxin-inhibitory effect of seeds diseased with Fusarium head blight in Example 1

Healthy grains and grains diseased with Fusarium head blight were screened from the crude wheat obtained in Example 1, and the DON contamination concentration of the whole grain powder thereof was analyzed by ELISA in the same way as shown in Example 1.

The results of the present test are shown in Table 2.

Table 2. DON contamination concentration of grains diseased with Fusarium head blight

	Treatment content			DON concentration (ppm)	
	Active ingredient A	Concentration	Control system	healthy grain	diseased grain
1	Potassium phosphite	0.038%	Conventional control	0.05	76.6
2		0.070%	Conventional control	0.04	44.7
3		0.112%	Conventional control	0.04	25.4
4			No control	0.04	75.6
5	Fosetyl	0.120%	Conventional control	0.96	11.0
6	Potassium phosphate	0.112%	Conventional control	0.06	86.9
7			Conventional control	1.72	90.0
8			No control	0.73	173.0

Note: The concentration of the active ingredient A is the concentration converted into P_2O_5.

Note: The grains colored scarlet or orange particular to Fusarium head blight and shrink grains were sorted as diseased grain, and all other grains were sorted as healthy grains.

The DON contamination concentration in healthy grains varied generally at a low level. On the other hand, in diseased grains with Fusarium head blight, DON was detected at an extremely high concentration, as expected. However, in crude wheat determined to be diseased, differences were observed in the DON contamination concentration. That is, in the plot treated with potassium phosphite, there was a concentration-dependent decrease in DON contamination depending on the amount of potassium phosphite applied. Therefore, it is obvious that applying potassium phosphite inhibits DON contamination in wheat regardless of the presence or absence, or the degree of the disease in cereals caused by Fusarium head blight, and further that

the inhibitory degree of DON contamination depends on the concentration of potassium phosphite applied.

Example 3: Combination use (1)

Wheat (cultivar: Haruyutaka) was seeded on April 23, 2002, and cultivated according to a conventional cultivating standard (Hokkaido, Agriculture Department, 1995) to establish test plots of 10 m^2 per plot (3 repeats). A suspension containing potassium phosphite and various fungicidal agents was prepared in a liquid mixture consisting of a spreading agent (Gramine S: supplied from Hokkai Sankyo Co., Ltd.) and water. By using potassium phosphite as compound A, 0.070% as P_2O_5, and 0.006 to 0.025% fungicidal agent for agri-horticulture for wheat as compound B, an aqueous solution containing the compounds was prepared and sprayed on leaves in the following growth stages at a rate of 100L/10a. That is, a first spray (June 24, heading date), a second spray (June 30, flowering stage), and a third spray (July 9) were performed. The harvest was performed on August 12 (50 days after the heading date) by harvesting the wheat from 3.4 m^2 in each plot. After harvest, the wheat was applied to a rice grader with 2.2 mm screen openings to sieve selected grains, which were then pulverized by a high-speed pulverizer to make whole grain powder. The whole grain powder was used as an analysis sample. The DON contamination concentration was analyzed using HPLC-UV in officially fixed methods set by the Minister of Health, Labor and Welfare. Quantitative analysis was performed by repeating the analysis three times, and the quantitative value was the average value thereof.

The preparation of analysis sample solutions and the analytical procedure are briefly described below.

- 85% acetonitrile was added to 50 g of the whole grain powder and vigorously agitated for 30 min, and subsequently sonicated for 10 min.

- After eliminating impurities with filter paper, the filtrate was purified using a pretreatment column MultiSep #227 to make an analysis solution.

- The test analysis solution was injected into high performance liquid chromatography, and DON was detected by ultraviolet rays.

- The DON concentration of each test solution was read out from a calibration curve made using DON standard solutions.

- The incidence rates of Fusarium head blight for panicles and spikelets were surveyed for 100 panicles in each plot.

The results of the present test are shown in Table 3.

Table 3. Combination use effects of potassium phosphite and various fungicidal agents

	Presence or absence of active ingredient A, potassium phosphite	Active ingredient B, Concentration of active ingredient	DON concentration (ppm)	Incidence rate for panicles (%)	Number of diseased spikelets/ panicle
1	×	Tebuconazole 0.020%	0.39	0.3	0.003
2	○		0.38	1.0	0.010
3	×	Metconazole 0.006%	0.34	0.7	0.007
4	○		0.10	1.0	0.010
5	×	Propiconazole 0.017%	1.01	1.0	0.010
6	○		0.72	2.7	0.027
7	×	Azoxystrobin 0.010%	4.65	2.3	0.027
8	○		0.96	2.0	0.023
9	×	Kresoxim-methyl 0.021%	1.05	0.3	0.003
10	○		0.51	0.7	0.007
11	×	Iminoctadine acetate 0.025%	1.04	1.7	0.017
12	○		0.20	3.0	0.030
13	○		0.77	4.3	0.050
14	Non-treatment		3.69	6.7	0.138

Note: potassium phosphite being 0.07% as P_2O_5; ○: treated, ×: untreated

Note: The incidence rates for panicles and spikelets were surveyed for 100 panicles in each test plot.

Example 4: Combination use (2)

Wheat (cultivar: Haruyutaka) was seeded on May 4, 2003, and cultivated according to a conventional cultivating standard (Hokkaido, Agriculture Department, 1995) to establish test plots of 10 m^2 per plot (3 repeats). A suspension containing potassium phosphite and various

fungicidal agents was prepared in a liquid mixture consisting of a spreading agent (Gramine S: supplied from Hokkai Sankyo Co., Ltd.) and water. By using potassium phosphite as compound A, 0.070% as P_2O_5, and 0.030 to 0.125% fungicidal agent for agri-horticulture for wheat as compound B, an aqueous solution containing the compounds was prepared and sprayed on leaves in the following growth stages at a rate of 100L/10a. That is, a first spray (July 1, flowering stage), a second spray (July 7), and a third spray (July 14) were performed. Harvesting was performed on August 25 by harvesting all the wheat in each test plot. After harvesting, by using a sample divider, the wheat was equally divided, and was applied on a rice grader with 2.2 mm screen openings to sieve selected grains, which were then pulverized by a high-speed pulverizer to make whole grain powder. The whole grain powder was used as an analysis sample. The DON contamination concentration was analyzed by ELISA in the same way as shown in Example 1.

Table 4. Combination use effects of potassium phosphite and various fungicidal agents

	Presence or absence of active ingredient A, potassium phosphite	Active ingredient B, Concentration of active ingredient	DON concentration (ppm)	Incidence rate for panicles (%)	Number of diseased spikelets/ panicle
1	×	Tebuconazole 0.020%	0.16	7.0	7.3
2	○		0.08	5.0	5.0
3	×	Propiconazole 0.017%	0.57	4.7	5.0
4	○		0.11	4.7	5.0
5	×	Azoxystrobin 0.010%	0.83	4.3	4.7
6	○		0.50	3.7	4.0
7	×	Trifloxystrobin 0.025%	0.35	4.3	5.0
8	○		0.12	3.0	3.0
9	×	Iminoctadine albesilate 0.030%	0.22	7.3	8.0
10	○		0.00	2.7	2.7
11	×	sulfur 0.125%	1.85	8.7	10.3
12	○		0.43	8.7	8.7
13	○		0.57	10.3	11.0
14	Non-treatment		2.15	20.4	23.8

Note: potassium phosphite being 0.070% as P_2O_5. ○: treated, ×: untreated

Note: The incidence rates for panicles and spikelets were surveyed for 100 panicles in each test plot.

In the same way as shown in Example 3, when used in combination with any fungicidal agents for agri-horticulture, both the incidence rate for panicles and the incidence rate for spikelets exhibited high values compared to the treatment alone, but DON contamination was inhibited.

It is worthy of special mention that the result in the plot applied with the treatment with compound A alone, where the disease rate of Fusarium head blight was high, but nonetheless, DON contamination was low.

Example 5: Effects on bacterial growth and toxin production (1)

An aqueous solution containing 5.600% potassium phosphite was prepared and soaked into gas-sterilized "Hokushin" wheat seeds , and the wheat seeds were subsequently inoculated with the Fusarium head blight pathogenic fungus in cereals, *Fusarium graminearum*, which has DON producing capacity, then cultured at 27°C. On days 7, 14, 21 and 28 in culture, the amount of Fusarium head blight pathogenic fungus in cereals and the amount of DON production in the wheat grains was analyzed.

The preparation of ergosterol sample analysis solutions and the analytical procedure are briefly described below.

- 80 ml of ethanol was added to 5 g of the culture, and pulverized using a high-speed pulverizer.

- The ethanol solution containing the pulverized culture was vigorously agitated for 30 min to extract ergosterol.

- After eliminating impurities with filter paper, a filtrate was concentrated under reduced pressure, dried, solidified and dissolved again in 10 ml of ethanol to make a sample analysis solution.

- The sample analysis solution was injected into high performance liquid chromatography, and ergosterol was detected by ultraviolet rays.

The preparation of DON sample analysis solutions and the analytical procedure are briefly described below.

- 80 ml of distilled water was added to 4 g of the culture, and pulverized using a high-speed pulverizer.

- The aqueous solution containing the pulverized

culture was vigorously agitated for 30 min to extract DON.

- A part of the extract solution was centrifuged, and the supernatant was used for the ELISA.

- According to the instruction manual in the ELISA kit, various reagents were added, and subsequently the absorbance of each test solution was measured.

- The DON concentration of each test solution was read out from a calibration curve made using a DON standard solution.

Table 5. Change over time of bacterial growth and DON production in wheat treated with potassium phosphite

Treatment	Analysis item	Days of culturing			
		7 days	14 days	21 days	28 days
Potassium phosphite 5.6%	DON(ppm)	ND.	ND.	ND.	ND.
	Ergosterol peak area value	17952	115921	372377	350972
Non-treatment	DON(ppm)	6.3	4.43	4.29	37.8
	Ergosterol peak area value	150453	271882	367236	559014

Note: ND means that the analytical value is less than the detection limit (0.222 ppm) of the ELISA kit.

The amount of ergosterol, which is an indicator of the amount of fungi, was continuously increased during culture in the non-treatment whereas it reached a plateau on the 21st day in the treatment with the potassium phosphite . With respect to the amount of DON production, in the non-treatment, DON production was initiated at an early phase in culture and remarkably increased on the 28th day. On the contrary, in the treatment with potassium phosphite, DON was not detected during the entire culture period. It is obvious that application of potassium phosphite has a high inhibitory effect on DON production

regardless of the presence or absence, or the degree of proliferation of Fusarium head blight (pathogenic) fungus in cereals.

Example 6: Effects on bacterial growth and toxin production (2)

An aqueous solution containing 0.056% to 2.800% potassium phosphite was prepared and soaked into gas-sterilized "Hokushin" wheat seeds , and the wheat seeds were subsequently inoculated with Fusarium head blight pathogenic fungus in cereals, *Fusarium graminearum*, which has DON producing capacity, then cultured at 27°C. On day 28 in culture, the amount of Fusarium head blight pathogenic fungus in cereals and the amount of DON production in wheat grains were analyzed by the same methods as used in Example 5.

Table 6. Effects of potassium phosphite concentration on fungal amount of Fusarium head blight pathogenic fungus and the amount of DON production

Treatment concentration	DON (ppm)	Ergosterol peak area
0.056%	1.60	1834861
0.112%	0.41	1769582
0.028%	ND.	1810454
0.560%	ND.	1921693
2.800%	ND.	85891
Non-treatment	46.1	3601748

Note: ND means that the analytical value is less than the detection limit (0.222 ppm) of the ELISA kit.

Considering the amount of ergosterol as an indicator of the number of fungi, the treatment with an aqueous solution containing 0.056 to 0.560% potassium phosphite

showed from a moderately better to a similar effect, and treatment with an aqueous solution containing 2.800% potassium phosphite a remarkable inhibitory effect against the fungus, when compared to the non-treatment (treatment with water alone). The treatment with the aqueous solution containing from 0.056 to 2.800% potassium phosphite showed significantly lower DON amounts when compared to the non-treatment. It is obvious that the application of potassium phosphite has a high inhibitory effect on DON production regardless of the presence or absence, or the degree of proliferation of Fusarium head blight (pathogenic) fungus in cereals.

Industrial Applicability

According to the invention, it has been demonstrated that an excellent inhibitory effect on mycotoxin contamination, particularly DON contamination, can be had regardless of the presence or absence or of the degree of Fusarium head blight disease in cereals although the controlling effect against Fusarium head blight pathogenic fungi in cereals is weakened by spraying one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester onto growing wheat. Also, it has been demonstrated that the amount of mycotoxin contamination is further reduced when compound A is used in combination with other fungicidal agents for agri-horticulture when compared to the use of a fungicidal agent alone.

Claims

1. A composition containing one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester as an active ingredient(s) when used to inhibit mycotoxin contamination in cereals.

2. The composition according to claim 1 wherein the compound A is an alkali metal salt or a polyvalent metal salt of phosphorous acid or phosphite ester.

3. The composition according to claim 1 wherein the compound A is an aluminium salt of tris(ethylphosphonate).

4. The composition according to claim 1 wherein the compound A is potassium phosphite.

5. The composition according to any one of claims 1 to 4 containing compound A and one or more fungicidal active ingredient(s) for agri-horticulture.

6. The composition according to claim 5 wherein the fungicidal active ingredient is selected from the group consisting of an inhibitor of sterol biosynthesis having a triazole skeleton, a methoxyacrylate based fungicidal agent, a fungicidal agent which causes destruction of a membrane lipid bilayer structure of fungi and sulfur.

7. The composition according to claim 5 wherein the active ingredient is selected from the group consisting of tebuconazole, metconazole, propiconazole, azoxystrobin, kresoxim-methyl, iminoctadine acetate, iminoctadine albesilate, trifloxystrobin and sulfur.

8. The composition according to claim 5 wherein the fungicidal active ingredient is azoxystrobin, iminoctadine acetate or iminoctadine albesilate.

9. The composition according to any one of claims 1 to 8 wherein the mycotoxin in deoxynivalenol.

10. A method for inhibiting mycotoxin contamination in cereals wherein a composition containing one or more compounds A selected from the group consisting of ammonium salts, primary to quaternary ammonium salts, alkali metal salts, alkaline earth metal salts and polyvalent metal salts of phosphorous acid and phosphite ester as an active ingredient(s) is applied to the cereals.

11. The method according to claim 10 wherein the compound A is an alkali metal salt or a polyvalent metal salt of phosphorous acid or phosphite ester.

12. The method according to claim 10 wherein the compound A is an aluminium salt of tris(ethylphosphonate).

13. The method according to claim 10 wherein the compound A is potassium phosphite.

14. The method according to any one of claims 10 to 13 wherein the composition contains a compound A and one or more fungicidal active ingredient(s) for agri-horticulture.

15. The method according to claim 14 wherein the fungicidal active ingredient is selected from the group consisting of an inhibitor of sterol biosynthesis having a triazole skeleton, a methoxyacrylate based fungicidal agent, a fungicidal agent which causes destruction of a membrane lipid bilayer structure of fungi and sulfur.

16. The method according to claim 14 wherein the fungicidal active ingredient is selected from the group consisting of tebuconazole, metconazole, propiconazole, azoxystrobin, kresoxim-methyl, iminoctadine acetate, iminoctadine albesilate, trifloxystrobin and sulfur.

17. The method according to claim 14 wherein the fungicidal active ingredient is azoxystrobin, iminoctadine acetate or iminoctadine albesilate.

18. The method according to any one of claims 10 to 17 wherein the mycotoxin is deoxynivalenol.

19. A cereal whenever produced by the method of any one of claims 10 to 18.

20. A composition according to any one of claims 1 to 9 substantially as herein described with reference to Examples 1 to 6, Tables 1 to 4 and/or 6.

21. A composition according to any one of claims 1 to 9 substantially as herein described.

22. A method according to any one of claims 10 to 18 substantially as herein described with reference to Examples 1 to 6.

23. A method according to any one of claims 10 to 18 substantially as herein described.

Fig.1

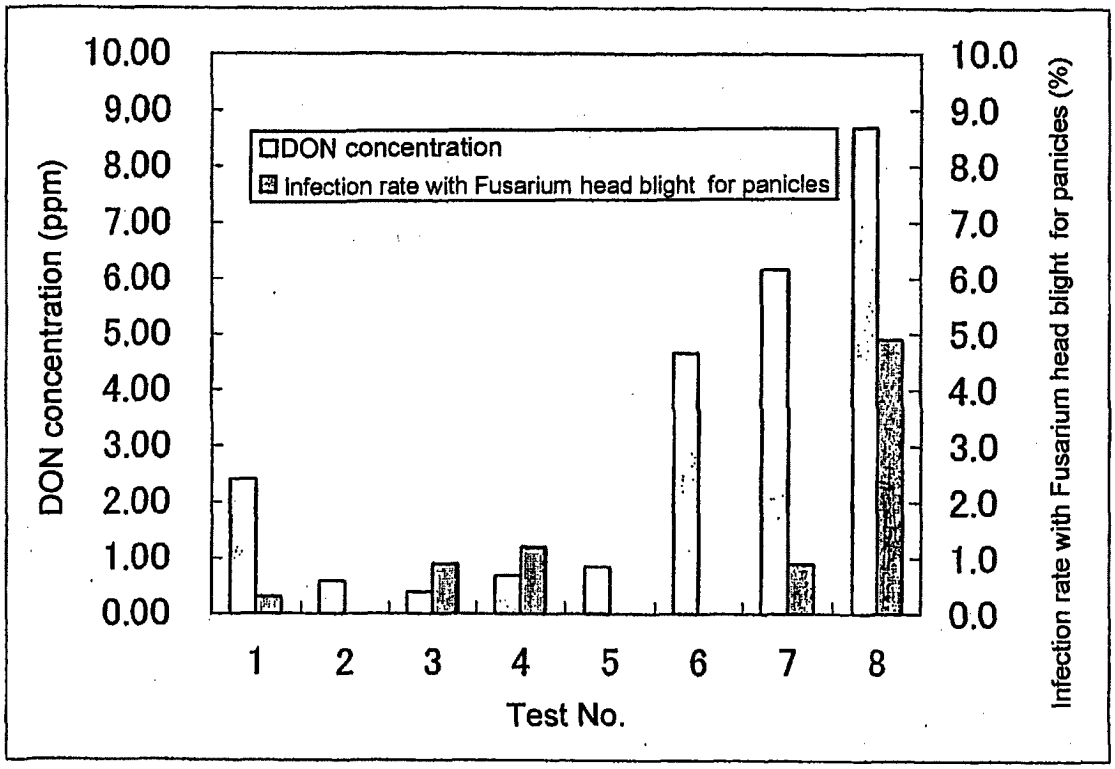

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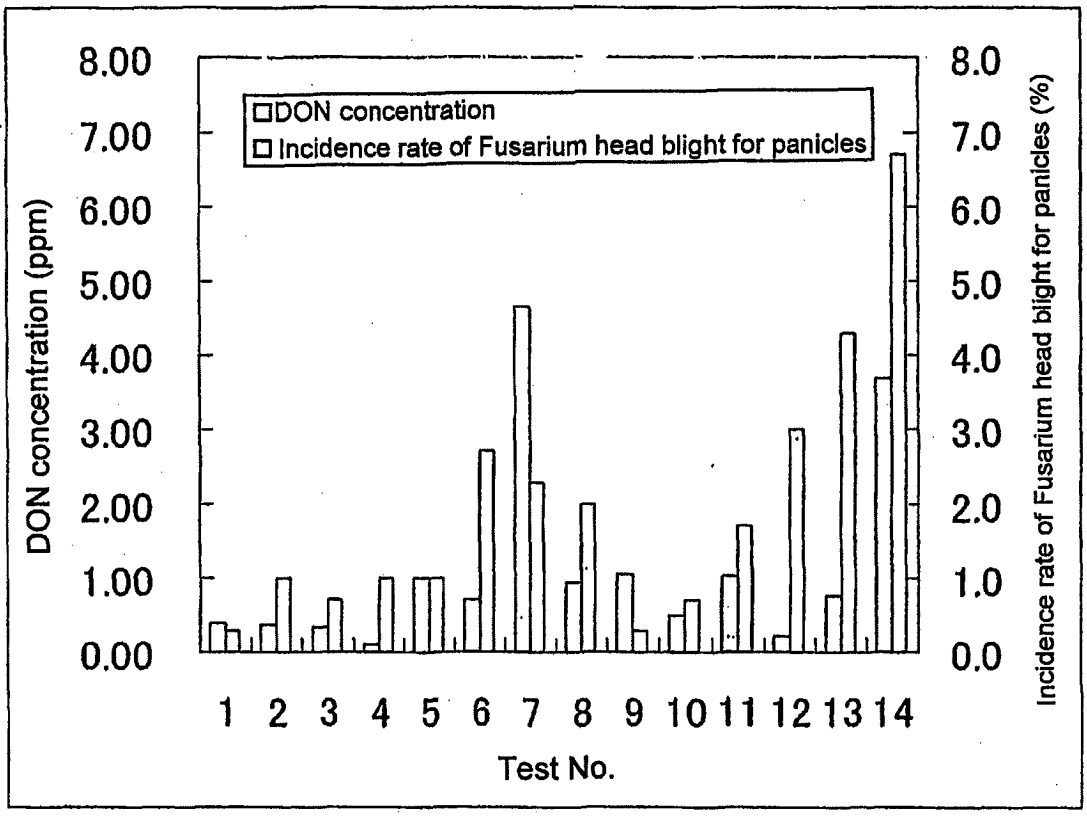
Fig.2



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